

Biodiversity of Fungi

Their Role in Human Life

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CHAPTER

6

***Phoma* Saccardo and Related Genera: Some New Perspectives in Taxonomy and Biotechnology**

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SUMMARY

The taxonomy of the genus *Phoma* Sacc. has been a matter of controversy. Majority of the mycologists used host alone as a criterion for identification and differentiation of *Phoma* and *Phoma*-like fungi. Consequently, a large number of these fungi were erected. The *Phoma* and *Phoma*-like fungi were studied in detail by many investigators and it was realized that many genera should be relocated on the basis of detailed morphological and cultural characteristics and ontogeny.

The present chapter is focused on the taxonomic problems associated with *Phoma* and *Phoma*-like fungi, criteria for identification and differentiation of these fungi, discussion about different sections of *Phoma*, diversity in Indian species of *Phoma*, new perspectives in *Phoma* taxonomy, integrated approach of speciation, biotechnological potential, particularly in the field of pharmaceutically active metabolites (antibiotics), mycoherbicides and agrochemicals, anthraquinones dyes, mutualistic association with *Taxus wallachiana*, future strategies to use molecular probes for the identification and differentiation of the species of *Phoma* and their potential in the field of biotechnology.

INTRODUCTION

The genus *Phoma* has had a complicated history since it was initially introduced more than 180 years ago. In the nineteenth and the first half of the twentieth century, according to Saccardo's (1878, 1884) concept, thousands of non-stromatic species of pycnidial fungi were described which produce hyaline conidia without septa. According to this system, the host-specificity and existence or lack of septum was emphasized. Generally, *Phoma* was used for those species which had 1-celled, hyaline conidia and grew on stems and branches of plants; also in the *Phyllosticta* genus classified similar species growing on leaves. The species having two-celled hyaline conidia and growing on stems were included in *Diplodina*, while species having similar conidia but growing on leaves were often placed in *Ascochyta*. A lot of *Phoma*-like fungi occur both on leaves and stems; however, pycnidia of much species contain both 1- and 2-celled conidia. A large number of almost 2000 'species' have been described and this is certainly due to the fact that these fungi are amongst the most ubiquitous microorganisms, occurring in a wide variety of ecological niches (Boerema, 1964; Sutton, 1980). As a result, there was chaos and a lot of *Phoma*-like fungi have synonymous in all four genera (Van der Aa and van Kesteren, 1971; Boerema and Dorenbosch, 1973).

The first modern studies on *Phoma* were carried out by Wollenweber and Hochapfel (1936) and Dennis (1946), introducing in vitro characters. This formed a basis for a more sophisticated and natural circumscription of the Saccardoan form-genera.

Since the early 1960s, Boerema and his co-workers and later Morgan-Jones and their co-workers have extended these concepts to a much larger number of *Phoma* taxa from a wider range of hosts (Boerema and Höweler, 1967; Dorenbosch, 1970; Boerema and Dorenbosch, 1973; Boerema, 1976; Boerema et al. 1977; Boerema and van Kesteren, 1981; Morgan-Jones and White, 1983; Morgan-Jones and Burch, 1987 a,b, 1988; White and Morgan-Jones, 1987 a,b; Morgan-Jones, 1988). In addition to the existing limited number of traditional morphological criteria, these authors introduced features such as accessory spore states (including sclerotia and chlamydospores), the ability to develop conidial septa, differences in growth rate on at least three culture media, formation of crystals in vitro, production of secondary metabolites and associated pigmentations and their changes by NaOH spot test.

On the basis of detailed studies of the type species of *Phoma* and *Ascochyta* (Boerema, 1964; Boerema and Bollen, 1975), numerous species or species groups were described with special reference to ecological and phytopathological features (Boerema et al. 1965, 1968, 1971, 1973, 1977, 1981; Boerema and Höweler, 1967; Boerema and van

Kesteren 1964, 1981; Dorenbosch, 1970; Boerema 1970, 1972, 1976, 1984, 1985, 1986, 1993; Boerema and Dorenbosch, 1970, 1973; Van der Aa and van Kesteren, 1971, 1979; Boerema and Loerakker, 1981, 1985; Morgan-Jones and White, 1983; Morgan-Jones and Burch 1987a,b, 1988; White and Morgan-Jones 1987a,b; Morgan-Jones, 1988; de Gruyter and Noordeloos, 1992; de Gruyter et al. 1993; Kövics et al. 1999).

Van der Aa (1973) revised the genus *Phyllosticta* and only 7% of the thousands of species described are in the amended generic concept. Finally, almost 50% of species had to be reclassified in *Phoma*, about 20% in *Asteromella*, and 5% in *Phomopsis* genera. The remaining 18% were placed among Coelomycetes (Sphaeropsidales, Melanconiales), some species in the Hyphomycetes (Moniliales) or even into Ascomycota.

The latest revised *Phyllosticta* monograph were published by van der Aa and Vanev (2002) with a dozen taxonomic novelties in *Phyllosticta*, and many species were redispersed in genera *Aposphaeria*, *Ascochyta*, *Asteromella*, *Cleistophoma*, *Coleophoma*, *Fusicococum*, *Microphaeropsis*, *Phoma* and *Phomopsis*.

The genus *Pyrenochaeta* was monographed by Schneider (1979) but a number of species had to be reclassified in the *Phoma* section to *Paraphoma*. The distinction from *Phoma* is mainly based on the acropleurogenous arrangement of the conidiogenous cells on elongated conidiophores and not by the presence of setae on the conidiomata (Van der Aa et al. 1990).

Boerema and co-workers (Boerema et al. 1965, 1968, 1971, 1973, 1977, 1981) abandoned the host or substratum as the primary criterion for *Phoma* species concepts and tried to define their taxa on stable morphological characteristics, both in vivo and in vitro, combined with cultural characteristics using standardized conditions. The presence of setae and the anatomy of the pycnidial wall are of importance. Taxonomic significance can also be attributed to the presence of characteristic dictyochlamydospores. For the delimitation of species or infraspecific taxa, variation in size and shape of pycnidia and conidia, and cultural characteristics have proved essential.

In 1977, Melnik published a comprehensive book on the genus *Ascochyta*, followed by Punithalingam (1979, 1988), who started a series of *Ascochyta* monographs on Monocotyledons. Sutton (1977, 1980) summarized his conception with numerous genera of Coelomycetes.

Looking at the rapid increase in claims of numbers of species, the following points are to be taken into consideration: Among more than 2000 described *Phyllosticta* species, only a few can be classified as true *Phyllosticta* Pers. ex Desm. About 1300 species of *Ascochyta* and *Diplodina* are described but only a few belong to true *Ascochyta* Lib. More

than 5000 species of *Phoma*, *Phyllosticta*, *Ascochyta* and *Diplodina* belong to *Phoma* Sacc. (Boerema and Bollen, 1975).

About 200 true *Phoma* taxa have been defined and recognized which can be divided into two large groups: (i) plurivorous fungi, generally saprophytic or weakly parasitic (opportunistic), mainly from temperate regions in Eurasia, but occasionally also found in other parts of the world (including areas with cool or warm climates); and (ii) specific pathogens of cultivated plants (Van der Aa et al. 1990).

The previous decade witnessed the publishing of contributions toward *Phoma* genus monographs (de Gruyter and Noordeloos, 1992; Boerema 1993, 1997; Boerema et al. 1994, 1996, 1997, 1999; de Gruyter, 2002; de Gruyter and Boerema, 2002; de Gruyter et al. 1993, 1998, 2002; Boerema and de Gruyter, 1998, 1999; Van der Aa et al. 2000) in *Persoonia* and *Mycotaxon* on the basis of the previous 30-year results of their research work. The new *Phoma* monograph (Boerema et al. 2004) fills the gap of practical manual to aid such identification with cultural description of 223 specific and infra-specific taxa, and classification of 1146 synonyms in various Coelomycetes genera.

The above-mentioned studies on taxonomical conceptions of *Phoma*, *Phyllosticta*, *Ascochyta*, *Pyrenochaeta* and some other genera of Coelomycetes became generally accepted (Schneider, 1979; Sutton, 1980; Johnston, 1981; von Arx 1981, 1987; Morgan-Jones and White 1983; Taber et al. 1984).

PHOMA AND ITS SECTIONS

The conidiogenesis and septa production of the lectotype species of *Phoma* Saccardo (1880), the ubiquitous saprophytic *Phoma herbarum* Westend; was studied in detail by Brewer and Boerema (1965), and later by Boerema and Bollen (1975) on some other *Phoma* and *Ascochyta* species. Coinidiogenesis of *Phoma* species show phialidic ontogeny; septum of conidia consist of three slimy layers. However, most conidia are 1-celled but secondary septa can be formed especially in vivo (0-95%); wall of the septate conidium is not thicker than that of the aseptate conidium (Boerema and Bollen, 1975; Boerema, 1997). Among general laboratory conditions (in vitro), most conidia remain 1-celled; in all the cases, the first conidium of a conidiogenous cell arises as a thick-walled papilla (Boerema, 1997).

Conidiomata are mainly smooth pycnidia but sometimes hyphal appendices or setae may occur.

Within *Phoma*, a number of sections are distinguished that form the basis for the arrangement of taxa. Pycnidial morphology is only helpful with respect to the delimitation of sections within the genus.

Presently, about ten sections are recognized; some of these are artificial, but others represent natural units (Boerema, 1985; Van der Aa et al. 1990; Boerema et al. 2004). Despite this heterogeneity, members of all sections are united in one common characteristic: that the majority of the hyaline conidia remain 1-celled in vitro.

The characteristics of the most common five sections are discussed by Van der Aa et al. (1990); nine of them, sect. *Phoma sensu stricto* (68 taxa), sect. *Peyronellaea* (16 taxa), sect. *Plenodomus* (32 taxa), sect. *Heterospora* (17 taxa), sect. *Macrospora* (9 taxa), sect. *Phyllostictoides* (41 taxa), sect. *Pilosa* (2 taxa), sect. *Paraphoma* (12 taxa) and sect. *Sclerophomella* (13 taxa) are the subjects of the published 'Contributions towards a monograph of *Phoma*' (de Gruyter and Noordeloos, 1992; Boerema, 1993, 1997; de Gruyter, 2002; de Gruyter and Boerema, 2002; de Gruyter et al. 1993, 1998, 2002; Boerema et al. 1994, 1996, 1997, 1999; Boerema and de Gruyter, 1998, 1999, Van der Aa et al. 2000). Diagrammatic representation of these sections has been shown in Fig. 6.1.

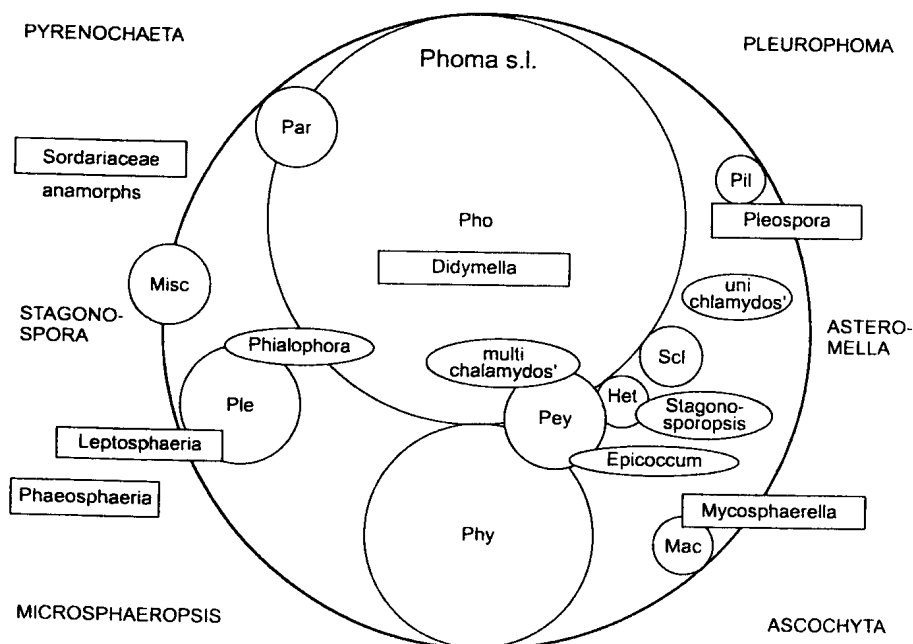


Fig.6.1 Diagrammatic representation of the different sections presently distinguished within the genus *Phoma* s.l. (after Boerema, 1997) Abbr.: Pho = sect. *Phoma*, Par = sect. *Paraphoma*, Pey = sect. *Peyronellaea*, Phy = sect. *Phyllostictoides*, Scl = sect. *Sclerophomella*, Ple = sect. *Plenodomus*, Mac = sect. *Macrospora*, Pil = sect. *Pilosa*, Het = sect. *Heterospora*, O with indication of connected teleomorphs = □, synanamorphs = O, and 'adjacent' or 'convergent' genera. The diameter of the circles reflects the relative species numbers; arrangement, touching and overlap of the circles indicates resemblance. Remark: The fungus does not contain the section "Miscellaneous" (with 14 taxa, Boerema et al. 2004)

DIVERSITY IN INDIAN *PHOMA*

The existing Indian species of *Phoma* have been claimed on the basis of host alone, and thus the importance of host specificity for the taxonomy of *Phoma* has been much emphasized and over estimated. The assumption that each host genus or species was colonized by a specialized *Phoma* species prompted many mycologists to ignore morphological characters when erecting new *Phoma* species. Usually, a morphological species may attack various host plants. For example, *P. exigua* has been reported by investigators on different hosts (Bhowmik and Singh, 1976; Raut, 1977; Kamal and Singh, 1979 and Rao and Thirumalachar, 1981). As a matter of fact, the criteria for identification should be such so that it should be possible to identify a *Phoma* species in case host identification is difficult, particularly when floral parts are lacking, or when the fungus is grown on artificial media.

Astonishingly, in the past, many new species have been erected from India, giving sole importance to the host (Pavgi and Singh, 1966; Bilgrami, 1963; Agarwal and Sahni, 1964; Dutta and Ghosh, 1965; Chandra and Tandon, 1965, 1966; Shreemali, 1972, 1973, 1978; Jamaluddin et al. 1975; Maiti et al. 1978; Rai and Misra, 1981; Agrawal and Misra, 1981). However, in the nineties, erection of species of *Phoma* was minimized (Borborua, 1990; Firdousi et al. 1990; Chile et al. 1992). Later on, morphological and cultural studies were also considered (Rajak, 1981; Rajak and Rai, 1983 a,b, 1984, 1985; Rai and Rajak, 1984, 1986, 1993; Rai, 2003).

NEW PERSPECTIVES IN *PHOMA* TAXONOMY

Several metabolites (e.g. antibiotic 'E') are also known to be specifically produced in pure cultures of *Phoma*. Crystals are formed by certain species in characteristic patterns, and pigments, often in connection with macrochemical reactions (NaOH spot test), are reliable criteria useful for rapid identification (Dorenbosch, 1970; Van der Aa et al. 1990; Noordeloos et al. 1993).

In addition to culture morphology, various metabolites and pigments provide potentially useful information for differentiating between taxa. Noordeloos et al. (1993) studied six *Phoma* and one *Ascochyta* species that produce characteristic dendritic crystals in pure culture. The chemical nature of the crystals has proved to be a specific character of the species involved. *Phoma andina* and *P. crystalliformis* (formerly considered varieties of the same species, and both pathogenic to potato) could be confirmed as distinct not only by morphological characters but also by secondary metabolite production. *P. andina* produces less distinct

'radicinin' crystals on oatmeal agar, while *P. crystalliniformis* produces 'brefeldin A' both on oatmeal and malt agar media. Three other *Phoma*-like fungi can now be better distinguished knowing the chemical constituents of the crystals: *P. medicaginis* forms 'brefeldin', whereas *P. pinodella* and *Ascochyta pinodes* form crystals of 'pinodellalide A and B'. Both *P. dorenboschii* and *P. arachidicola* also form crystals of 'pinodellalide A and B'.

Monte et al. (1991) published a vast amount of biochemical data based on methods frequently applied to bacteria, yeast and hyphomycetes taxonomy, contributing integrated physiological and biochemical features, supporting some traditional morphological approaches to the systematics of *Phoma* genus.

Isozyme profiles (e.g. acetyl esterase) can be an useful additional feature to the delimitation in *Phoma* (Monte et al. 1990, 1991; Kövics and de Gruyter, 1995; Saniewska and Prus-Glowacki, 1998; Kövics, 2004). Genetic differentiation by molecular biological analyses has also been started (Abeln et al. 2002)

INTEGRATED APPROACH OF SPECIATION

The erection of new species of *Phoma* in India has been based mostly on the host and sometimes on the shape and size of pycnidia and pycnidiospores. This criterion may be misleading, particularly when it is difficult to identify the host or when the substrate is other than plant/animal. Taking this into consideration, there has been an awakening among the fungal taxonomists in the eighties and the nineties concerning the erection of new species. The best way is to study the so-called new species in pure culture and follow an integrated approach based on cultural, morphological, physiological determination of ontogeny, biochemical evaluation or, in ambiguous cases, molecular approaches should be applied for identification and differentiation of a particular species (Rai, 1981; Monte et al. 1991).

Rai (1981) studied Indian species of *Phoma* in pure culture on malt-agar, oat-agar and rice-agar, and proposed a key to the identification and differentiation of different species of *Phoma*.

Application of molecular-biological methods in addition and/or completion to the traditional morpho-physiological methods in *Phoma* and *Phoma*-like fungi taxonomy still await introduction.

Phoma can be rapidly detected by polymerase chain reaction (PCR). Rollo et al. (1990) reported such a method for fast detection of *Phoma tracheiphila*. PCR-based markers for the identification of *Phoma* were also tried for *P. sclerotoides* infecting alfalfa (Larsen et al. 2002).

BIOTECHNOLOGICAL POTENTIAL

Various species of the genus *Phoma* produce several phytotoxic secondary metabolites, some of them are as follows:

Phoma lingam

The fungus produces two important phytotoxic compounds, viz., 'sirodesmin PL' and 'deacetylsirodesmin PL' (Ferezou et al. 1977). 'Phomamide', a phytotoxic intermediate compound, has been extracted from this fungus during 'sirodesmin' synthesis (Vining and Wright 1977; Curtis et al. 1977; Ferezou et al. 1980 a,b; Stoessl, 1981).

Phoma herbarum

'Brefeldin A', a component with pronounced phytotoxic property, has been isolated from several fungi, including this fungus. Structure and synthetic pathways of the compound have been extensively discussed in many publications (Sigg, 1964; Suzuki et al. 1970; Weber et al. 1971; Mabuni et al. 1979). Pandey et al. (2002) reported severe phytotoxicity of cell-free culture filtrate (CFCF) obtained from *P. herbarum* FGCC# 3 and 4 against *Lantana camara*. They recorded severe chlorosis, curling and finally a complete collapse of leaves within 48 hours of treatment. Toxic metabolites produced by FGCC# 3 are thermostable. Compound isolated with benzene solvent showed maximum phytotoxicity. Recently, Vikrant (2002) reported pronounced phytotoxicity of CFCF of *P. herbarum* FGCC#75 against *Parthenium hysterophorus*.

Phoma exigua

Rothweiler and Tomm (1966, 1970) purified and characterized 'phomin' and 'dehydrophomin' from the culture filtrate of *P. exigua* var. *exigua* (Desm.) Boerema. Other phytotoxic cytochalasins, viz., 'deoxaphomin', 'proxiphomin', 'protophomin' and 'cytochalasin A, B', have also been extracted from this pathogen (Binder and Tomm 1973; Scott et al. 1975; Rothweiler and Tomm 1966). 'Cytorhalasin A, B, F, T, U, V', 'assochalasin', and 'deoxaphomin' have been extracted from *Phoma exigua* var. *heteromorpha* (Capasso et al. 1991; Evidente et al. 1992). Vurro et al. (1997) have extensively reviewed the technological and biological aspects of these compounds.

Phoma sorghina

This fungus incites leaf spot infection in pokeweed (*Phytolacca americana* L.). Several phytotoxins, viz., diphenyl ether, epoxydon, desoxyepoxydon, phyllostine, 6-methyl salicylate ether have been produced by the pathogen. Among these, the epoxydons have shown very high broad spectrum toxicity to both mono- and dicotyledonous weed species (Venkatasubbaiah et al. 1992; Abbas and Duke, 1997).

Phoma macdonaldii

'Zinniol', a broad spectrum phytotoxin, has been isolated from this pathogen which is responsible for leaf and stem blight and withering of cut seedlings of many plants (Sugawara and Strobel 1986). The toxin binds to specific binding sites and is associated with the stimulation of Ca^{++} uptake by the affected cells at 0.1-1.0 μm levels. Calcium-regulated cell processes may be disrupted by this toxin. Its relative simple structure could be the basis for herbicide discovery studies (Abbas and Duke, 1997).

Phoma tracheiphila

Culture filtrate of this pathogen is very rich in secondary metabolites with high phytotoxic properties (Yoder, 1980; Gentile et al. 1992; Nachmias et al. 1977, 1979).

Phoma foveata

'Pachybasin', a phytotoxin has been isolated from this pathogen by Strange (1997).

It is evident from the above discussions that *Phoma* spp. could be a source for the novel agents against many weed problems, as they have both mycoherbicidal and biorational properties. Looking at the known species, the number associated with weeds is very small. This might be because of ignorance of weed pathogens. Mycologists as well as plant pathologists in the past have given more attention to economically important plant diseases.

Therefore, greater attention towards potential herbicides of *Phoma* is needed.

PHARMACEUTICALLY ACTIVE METABOLITES

There are some important antibiotics produced by some species of *Phoma* (Pearce 1997; Singh et al. 1997; Baxter et al. 1998). Pharmaceutically active metabolites 'squalestatin-1' (S1) and

'squalenol-2' (S2) are produced by a *Phoma* sp. (IMI 332962) (Baxter et al. 1998). Singh et al. (1997) reported the production of some antitumour agents by *Phoma* sp. (MF 6118), including 'fusidienol-A', which is the second member of the fusidienol family of inhibitors to possess a novel tricyclic oxygen-containing heterocycle with a 7/6/6 ring system. 'Equisetin' and 'phomasetin' obtained from species of *Phoma* are useful against AIDS (Singh et al. 1998). They showed HIV virus integrase inhibition. Some marine microorganisms, including *Phoma*, are reported to possess antibiotic activities (Sponga et al. 1999). The marine species of *Phoma* exhibited activities against *Enterococcus faecium*, *Escherichia coli*, *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus* (Table 6.1).

MYCOHERBICIDES AND AGROPHYTOCHEMICALS

Weeds are serious problems not only in agricultural and forestry production, but also pose several major problems to human and animal health all over the world. Synthetic chemical herbicides have been the mainstay for weed control practices since the end of World War II and are undoubtedly responsible for much of the unparalleled increased crop productivity that has occurred during this period. The high costs involved in developing and registering chemical herbicides and recent trends in environmental awareness have prompted researchers to investigate alternative systems of weed control. Ideally, such a system would control target weeds at or near the same levels as that achieved with chemical herbicides while at the same time not poisonous for either the environment or non-target organisms (Boyette and Abbas, 1995; Pandey et al. 2001).

Weed control using plant pathogens, especially fungal pathogens, as effective alternatives to chemical herbicides have gained significant attention and momentum in the 1970s. The century-old concepts in weed control and plant disease epidemiology were successfully put to text and some economically important weeds were controlled by fungal pathogens using classical and mycoherbicidal strategies. With the advancement in the knowledge, biorationals and integrated management strategies have also come into the picture. Biological, technological and economical perspectives of various strategies have been extensively reviewed in several publications (Auld, 1990; Abbas and Duke, 1995, 1997; Boyette and Abbas, 1995; Charudattan, 1991, 1996; Hasija et al. 1994; Hoagland, 1990 a,b, 1999, 2001; Pandey, 1999, 2000; Pandey et al. 1990, 1996a,b, 1997, 2001; Saxena and Pandey, 2000, 2001).

Phomas are cosmopolitan fungi, their species occur on a wide variety of substrates ranging from various parts of plants, soil, water, air and even humans and animals. Several species of the genus live as parasites or

Table 6.1 Antibiotic production by some species of *Phoma*

<i>Phoma</i> sp.	Active chemical/ product	Reference
<i>Phoma exigua</i> var. <i>exigua</i>	Antibiotic E (antibacterial and antifungal) Cytochalasin B	Boerema and Höweler (1967) Binder and Tomm (1973)
<i>Phoma pigmentivora</i>	LL-D253 alpha	McIntyre et al. (1984)
<i>Phoma</i> sp.	Phomactin A	Sugano et al. (1991)
<i>P. exigua</i> var. <i>heteromorpha</i>	Cytochalasin F	Capasso et al. (1991)
<i>Phoma</i> sp.	Squalestatin (anti-infective agents)	Dawson et al. (1992) Sidebottom et al. (1992); Pearce (1997)
<i>Phoma</i> sp.	Squalestatin 1, 2 (S1, S2)	Baxter et al. (1992)
<i>Phoma</i> sp.	Antiviral agent (against HIV) Equisetin and Phomasetin	Singh et al. (1998)
<i>Phoma</i> sp.	Antitumour (Fusidienol A)	Singh et al. (1997)
<i>Phoma</i> sp.	Antibiotic, antitumour, RKS-1778	Takeya et al. (1997)
<i>Phoma</i> sp.	Antitumour (Phomacins)	Alvi et al. (1997)
<i>Phoma</i> sp.	Pesticide (Octahydronaphthol derivative MK8383, Patent)	Wakui et al. (1999)
<i>Phoma</i> sp.	Antiviral agent (against HIV) Equisetin	Hazuda et al. (1999)
<i>Phoma</i> sp.	Antibiotic activities	Sponga et al. (1999)
<i>Phoma</i> sp.	Ras- Farnesyltransferase Inhibitor	Ishii et al. (2000)
<i>Phoma</i> sp.	Squalastatin S1	Aldred et al. (2000)
<i>Phoma</i> sp. (NRRL 25697)	Phomadecalins A-D and Phomapentenone A	Che et al. (2002)
<i>Phoma</i> sp. (Q60596)	Antifungal antibiotic YM-202204	Nagai et al. (2002)

saprophytes on plants and often produce distinct disease symptoms, viz., leaf spots, lesions on stems, fruits or even on roots (tubers), damping off, die-back, seed and fruit rots, seedling blights, etc. It is surprising that despite the excellent phytopathogenic potential shown by various species or varieties of the genus, their mycoherbicidal potential has been neglected. Only a few scanty attempts have been made to evaluate them as mycoherbicides. Heiny (1990) isolated a highly host-specific strain of *Phoma proboscis* from diseased parts of field bindweed (*Convolvulus arvensis*). Heiny and Templeton (1991) have reported very high mycoherbicidal effects when the agent was applied to the seedlings of the weeds and atmospheric temperature ranged from 16-28°C and more than 9 hrs dew period. Heiny (1994) have extensively evaluated the compatibility of synthetic herbicides for integration with the mycoherbicidal agent.

Rajak et al. (1990) isolated a strain of *P. herbarum* from diseased leaves of *Parthenium hysterophorus* L. collected from central India. The fungus applied in 2.3×10^2 spores/ml caused more than 90% inhibition in seed germination, seedling mortality and leaf damage followed by reduction in height of *Parthenium* weed plants (Pandey et al. 1991). Pandey and Pandey (2000) recovered three strains of *P. herbarum* (LC # 32, 37, 39) from diseased leaves and stem of *Lantana camara*. All the three strains incite severe infection in the weed, specially at the seedlings stage (Pandey, S.K. 2000). Recently, Pandey S.K. (2002) also isolated a strain of *P. herbarum* FGCC # 70 from disease seedlings of an invasive weed, *Hyptis suaveolens* (L.) Poit, recording very high mycoherbicidal potential when the seedlings were treated with 2.0×10^5 spores/ml suspension at $28 \pm 1^\circ\text{C}$, 24 hr dew period. Several other species, viz., *Phoma campanulata* on *Cassia fistula* (Rajak and Rai, 1982), *P. exigua* on *Sesamum indicum* (Singh and Agarwal, 1973), *P. eupyrena* on *Achyrenthus aspora* (Khanna and Chandra, 1977), *P. glomerata* on *Parthenium hysterophorus* (Padmbai, 1976), *P. lantanae* on *Lantana camara* (Singh and Agarwal, 1974), *P. palmarum* on *Calotropis procera* (Khanna and Chandra, 1977; Kamal and Singh, 1979), *P. tridocis* on *Tridax procumbens* (Wehmeyer, 1964), *P. herbarum* var. *ipomoeae* (Kamal and Singh, 1979), and *P. euphorbiae* on *Euphorbia hirta* (Rangaswami et al. 1970) have been reported from various parts of India.

Excellent pesticide activity was reported against some phytopathogenic fungi by the octahydronaphthol derivative MK8383 of a *Phoma* sp. (Wakui et al. 1999). Club root of cruciferous plants can be controlled by *Phoma glomerata* and its product epoxydon (Arie et al. 1998, 1999). Raymond et al. (2000) reported that *P. glomerata* possesses the ability to colonize and finally inhibit the growth of powdery mildew on oak and, thus, can be used as a biocontrol agent. In fact,

powdery mildews are common plant pathogens, which can be easily identified by formation of profuse white powder-like conidia and mycelia. For biological control of powdery mildews, only one fungus *Ampelomyces quisqualis* Ces. as a hyperparasite is known. Therefore, enormous opportunities and possibilities exist to utilize this mycoparasitic fungus as a potential biocontrol agent. Pandey S.K. (2000) evaluated the mycoherbicidal potential of indigenous fungal pathogens against *Lantana camara* L., which is an obnoxious Indian weed. He isolated about 40 fungi from different parts of Madhya Pradesh. Out of these isolates, the maximum efficacy was shown by *Alternaria alternata* followed by *Phoma multirostrata*. The latter is thermophilic and originally reported from India by Mathur and Thirumalachar (1959). It is widely distributed in subtropical, tropical regions and warm greenhouses.

ANTHRAQUINONES DYES

Earlier, all colourants were obtained from natural sources: plants, lichens, insects or shellfish (Hobson et al. 1997). Among these, anthraquinones were predominant, as exemplified by red dye kermes from the insect *Kermococcus ibicish* (Thompson, 1971). Natural dyes are better than the manmade dyes in fastness and brilliance (Taylor, 1986). Today, synthetic anthraquinone dyes comprise the second-most important group of organic colorants.

The chemical synthesis of anthraquinones require using strong acids at high temperature and heavy metal catalysts, as a consequence of which, environmentally hazardous effluents and byproducts are produced. With increasing awareness of the environment degradation by industry, the disposal of industrial effluent is becoming more expensive and regulated strictly. A return to dyes extracted from molluscs, insects, fungi or plants grown in their environment is not the intention. These methods are not commercially practical and can, in many ways, be regarded as environmentally unfriendly and totally impractical. For example, 150, 000 insects reared on 0.16 hectares of cactus plants are required to produce 1 kg of cochineal dye. In order to obtain the dye, the insects are put in bags and dried slowly by exposure to the sun for a prolonged period. However, a microbial source cultivated in bioreactors is by no means out of question.

There are many fungi which produce anthraquinones as secondary metabolites. Fungal anthraquinones are polyketide-derived secondary metabolites that occur widely in many genera of fungi. Compared with the commercially available hydroxyanthraquinones, most possess an additional methyl substitution in position three, e.g. emodin. This allows a study of the effect of such a group on the dyeing properties of dyestuffs

derived from them. A fungal anthraquinone cynodontin (1,4,5,8-tetra hydroxy-3-methylantraquinone) was produced in sufficient purity to allow it to be transformed using a simple chemical step to a dye product. This was then compared with a commercially available close analogue (Hobson et al. 1997).

Phoma exigua Desm. produces pigments. Bick and Rhee (1966) have reported that *P. exigua* var. *foveata* (= *P. foveata*) contains many anthraquinone pigments, such as pachybasin, chrysophanol, emodin and phomarin. In acidic condition, this complex of pigments becomes yellow and in alkaline conditions, red. This character is based on an ammonia test described by Logan and Khan (1969). On malt-agar, *P. exigua* var. *foveata* (= *P. foveata*) gives a pinkish colour after exposure to ammonia. This is due to reaction of diffusible anthraquinones and their reaction with ammonia. In old cultures, anthraquinone pigments crystallize out as yellow-green crystals. Tichelaar (1974) found that the fungicide thiophanate-methyl accelerates and increases the crystallization process of the pigments.

Both *P. exigua* var. *exigua* and *P. exigua* var. *inoxydabilis* produce 'cytochalasin B', which are also known as 'phomine' (Bousquet and Barbier, 1972; Scott et al. 1975).

Some isolates of *P. exigua* var. *foveata* (= *P. foveata*) produce the antibiotic 'E-metabolite' similar to isolates of the ubiquitous *P. exigua* var. *exigua* (Boerema and Höweler, 1967). It is a colorless substance and can be easily demonstrated in cultures by sodium hydroxide test. On application of a drop of sodium hydroxide to the margin of colonies on malt agar, oxidation takes place and pigment alpha converts into pigment beta. Pigment alpha is red-purple at pH <10.5 and blue-green at pH >12.5. Pigment beta is yellow at pH <3.5 and red at pH >5.5.

Some other species of *Phoma* also produce anthraquinones and 'phomalgin A' (Pedras et al. 1995), pigments with commercial potential.

MUTUALISTIC ASSOCIATION WITH *TAXUS WALLACHIANA*

Endophytes are microbes living in the tissue of a macrophyte by developing a mutualistic relationship (Carroll, 1986, 1988). The endophytes cannot be considered as saprophytes since they are associated with living tissues, and may in some way contribute to the well being of the plant (Yang et al. 1994). There are many endophytic fungi which form a mutualistic association with *Taxus wallachiana* (Nepalese yew). The fungus primarily lives in the intercellular spaces of the host tissues acquiring support, protection and food from the nutrient-rich

phloem of *T. wallachiana*, while producing one or more antibiotics which provide protection from bacterial infection to the tree host. *Phoma* sp. lives in the intercellular spaces of tissues in the phloem, cambial region of the tree, and produces two antibiotic substances: altersolanol A and 2-hydroxy-6-methylbenzoic acid (Yang et al. 1994).

CONCLUSIONS AND FUTURE PERSPECTIVES

The taxonomy of *Phoma* has long been a matter of controversy due to slight differences in the size of pycnidia and pycnidiospores of the allied genera. However, this problem has now been solved by Boerema and co-workers.

A practical and more reliable way for authentic identification of *Phoma*- and *Phoma*-like fungi is to study the so-called new species in pure culture. An integrated approach based on cultural, morphological, physiological, determination of ontogeny, and biochemical evaluation should be applied. In ambiguous cases, molecular markers should be used for genus/ species differentiation. Moreover, various metabolites and isozyme profiles can be useful as additional features for the delimitation in *Phoma*. PCR (Polymerase Chain Reaction) technology may be applied for species differentiation.

Finally, the potential of *Phoma* can be applied for the production of antibiotics, agrochemicals and herbicides. The pigment-producing species offers enormous opportunities in the field of textile industry as natural and eco-friendly colouring agents. The endophytic species can be explored for their antibiotic production and should be studied for growth promotion.

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